Remarks

Claim Objections

Claims 1, 9 and 10 were objected to for informalities. Applicants respectfully traverse this objection to the extent that it is applied to the claims as amended.

Claim 1 has been amended to correct a typographical error to recite "less than 24 hours."

Claim 9 has been amended to define that the bacterial strain expresses a homologous nuclease gene which has been modified to enhance nuclease activity. Support for this amendment can be found in the specification at least at page 4, lines 9-16.

Claims 1, 9 and 10, as amended, have corrected these informalities.

Rejection Under 35 U.S.C. § 112, first paragraph

Claims 1-10 were rejected under 35 U.S.C. § 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the art that the inventor had possession of the claimed invention. Applicants respectfully traverse this rejection to the extent that it is applied to the claims as amended.

The Legal Standard

The general standard for the written description requirement is that "a patent specification must describe the claimed invention in sufficient detail that one skilled in the art can reasonably conclude that the inventor had possession of the claimed invention." *See* M.P.E.P. § 2163(I). All that is required is that the specification provides sufficient description to **reasonably** convey to those skilled in the art that, as of the filing date sought, the inventor was in possession of the claimed invention. *Union Oil of California v. Atlantic Richfield Co.* , 208 F.3d 989, 997, 54

U.S.P.Q.2d 1227, 1232 (Fed. Cir. 2000); *Vas Cath*, 935 F.2d at 1563-64. An applicant may show possession of the claimed invention by describing the claimed invention with all of its limitations using such descriptive means as words, structures, figures, diagrams, and formulas that fully set forth the claimed invention. *Lockwood v. American Airlines, Inc.*, 107 F.3d 1565, 1572, 41 USPQ2d 1961, 1966 (Fed. Cir. 1997). As noted in a recent decision by the Board of Appeals and Interferences, the written description requirement does not require a description of the complete structure of every species within a chemical genus. (*see Utter v. Hiraga*, 845 F.2d 993, 998, 6 U.S.P.Q.2d 1709, 1714 (Fed. Cir. 1988), stating "A specification may, within the meaning of 35 U.S.C. § 112, para. 1, contain a written description of a broadly claimed invention without describing all species that claim encompasses.").

An adequate written description of the invention may be shown by any description of sufficient, relevant, identifying characteristics so long as a person skilled in the art would recognize that the inventor had possession of the claimed invention. *Id.*, citing *Purdue Pharma L.P. v. Faulding Inc.*, 230 F.3d 1320, 1323, 56 USPQ2d 1481, 1483 (Fed. Cir. 2000); *Pfaff v. Wells Electronics, Inc.*, 55 U.S. at 66, 119 S.Ct. at 3 11, 48 USPQ2d at 1646 (1998).

In a recent decision by the Board of Patent Appeals and Interferences, the Board warned that it is an improper analysis to determine that the claims are directed to an invention which is broader than that which is described in the specification since the <u>written description is determined from the perspective of what the specification conveys to one skilled in the art</u> citing *In re GPAC Inc.*, 57 F.3d 1573, 1579, 35 USPQ2d 1116, 1121 (Fed. Cir. 1995) and *Vas Cath*, 935 F.2d at 1563-64. Thus the Board re-emphasized that the specification need not always spell

out every detail; only enough "to convince a person of skill in the art that the inventor possessed the invention and to enable such a person to make and use the invention without undue experimentation." *LizardTech Inc. v. Earth Resource Mapping, Inc.*, 424 F.3d 1336, 1344-34, 76 USPQ2d 1724, 1732 (Fed. Cir. 2005).

Although the "written description" requirement is a separate requirement from the "enablement" requirement, **if the enablement requirement has been met, it is difficult for the Examiner to assert that the written description requirement has not similarly been met.** The Federal Circuit recently expressed this in *LizardTech Inc. v. Earth Resource Mapping, Inc.*, stating "A recitation of how to make and use an invention across the full breadth of the claim is ordinarily sufficient to demonstration that the inventor possesses the full scope of the invention and vice versa." *LizardTech Inc. v. Earth Resource Mapping, Inc.*, 424 F.3d 1336, 1343, 76 U.S.P.Q.2d 1724, 1732 (Fed. Cir. 2005).

Analysis

Genetic engineering of bacteria has been practiced for decades, well before genetic engineering of mammalian cells and animals. Commercial fermentation of bacteria to produce amino acids and other products (including beer and bread and yogurt) has long been routine. Mutagenesis of bacteria and screening for a particular characteristic, such as antibiotic resistance, or increased production or tolerance to a product such as alcohol, is totally routine. One does not need to know the sequence of a gene to mutate the gene, nor the active site of the gene product, to screen for a desired property. All that is required is that one expose the bacteria to a known mutagen, then subject the bacteria to a screen for whatever is the desired product. In the present

case, the desired product is increased production and secretion of a nuclease, or production and secretion of a nuclease with more activity.

Bacterial strains, such as *Ralstonia*, *Aeromonas*, *Azotobacter*, *Burkholderia*, *Comamonas*, *Methylobacterium*, *Paracoccus*, *Pseudomonas*, *Rhizobium*, and *Zooglea*, have been sold by the American Type Culture Collection in Rockville, MD and used in school laboratories and commercial fermentation facilities for many years. All are well known to be amenable to typical manipulations of bacterial genetics, allowing the use of broad host range cloning vectors as transforming vehicles for a nuclease gene of interest (see at least the paragraph bridging pages 7 and 8).

Suitable nuclease genes were well known and described in the literature as of the date of filing of this application. Specific sources of suitable nucleases are taught at page 6, lines 4-13, and can be obtained and produced by using well established methods in the art, such as PCR and primers complementary to the sequence encoding the nuclease using information obtained from publicly available databases. Examples of such sequences are disclosed for many strains in GenBank (see at least page 6, lines 4-13; and page 7, lines 15-22). It is very common in the art to use degenerate primers, based upon known sequences, in PCR methods to isolate genes encoding proteins of desired function. Once the nuclease gene has been isolated, common genetic manipulation allows for its integration into a microbial strain (see at least page 7, lines 8-10).

The Examiner has presented no evidence to the contrary. No literature support has been provided that suitable strains or nuclease genes were not known, available, and could be routinely manipulated.

The application contains examples demonstrating that one can genetically engineer known strains of bacteria with known nuclease genes to make a product as claimed. Moreover, these strains can be screened for the desired characteristics with no further information than a simple assay for nuclease activity, or the desired viscosity of the cell lysate. Neither requires more than routine experimentation. **Example 1**, describes isolation of a suitable nuclease gene (page 11, line 30 to page 12, line 20); **Example 2**, construction of a vector to insert the nuclease gene into a *P. putida* bacteria (page 12, line 21 to page 13, line 16), screening for nuclease expressing clones (12,000 random integrants; 1500 colonies screened; 35 nuclease expressing clones; 9 secreting nuclease); **Example 3**, screening of *R. eutropha* bacterial strains for secretion of nuclease (1/10 produced nuclease in the periplasm) (page 14, lines 16-23). **Table 1** on page 15 shows the amount of nuclease secreted into the periplasm for six strains, of which three are high producers and one very high (MBX 979). **Example 6**, demonstrates the actual isolation of products from cell lysates from an engineered, screened bacterial strain, MBX 985, and a non-engineered strain (page 16, line 19 to page 17, line 5). The engineered strain produced the same low viscosity of the cell lysate that chemical treatment was required to obtain from the non-engineered strain.

The Examples clearly show that a high level of nuclease expression in the constructed strains is required to generate commercial levels of product (for example polyhydroxyalkanoate).

This is quite different from what is observed under laboratory conditions. For example, the claims are directed to fermenting bacterial strains capable of growth to cell densities of at least 50g/l and producing polyhydroxyalkanoates to levels of at least 40% of dry cell weight. The Examples illustrate fermenting bacterial cell cultures grown to a cell density of 200 g/l in 20 L fed-batch cultures, clearly indicating the need for high level nuclease expression in order to sufficiently reduce lysate viscosity and enhance product recovery (see **Example 6**). The high level of expression of the nuclease is reflected in **Table 2**, wherein the viscosity of each batch lysate is comparable to wild type cultures supplemented with BENZONASE™ at 10 microliters/L per culture.

Although Applicants believe the examples demonstrate that different bacterial species can be utilized, as well as methods of screening, M.P.E.P. 2164.02 clearly states, in part,

> "lack of working examples or lack of evidence that the claimed invention works as described [for all species] should never be the sole reason for rejecting the claimed invention on the ground of lack of enablement....to make a valid rejection, one must evaluate all of the facts and evidence and state why one would not expect to be able to extrapolate that one example across the entire scope of the claims."

The examiner has failed to provide any evidence or reasoning as to why those skilled in the art would not extrapolate from the actual examples in the application to other strains of bacteria or other nuclease genes. Mutagenesis of bacterial strains, nuclease activity assays, PCR isolation of nuclease genes from chromosomal DNA, PCR isolation of nuclease genes from DNA utilizing knowledge obtained from sequences already disclosed as GenBank reference

numbers, cell lysis methods to render accessible product and nuclease (if periplasmically localized), and, in general, what is already known about product recovery from bacterial strains, are all methods and relevant subject matter taught in the present specification and readily realized by one of ordinary skill in the art as commonplace in the field. Applicants respectfully submit that in view of these disclosed methods and what is already known, one of skill would have no problem isolating nuclease genes and transforming a fermenting bacterial host strain.

The Examiner has provided no rationale for why the specification fails to provide a sufficient written description for a fermenting bacterial strain that secretes a nuclease and is capable of growth to cell densities of at least 50g/l, a fermenting bacterial strain that secretes a nuclease and produces polyhydroxyalkanoate to levels of at least 40% of its dry cell weight, a fermenting bacterial strain that secretes a nuclease and is used in an aqueous process to manufacture poly(3-hydroxyalkanoates) granule suspension which is essentially free of nucleic acids, and a fermenting bacterial strain that secretes a nuclease and is used in a process for making specific polysaccharides, as is actually demonstrated by example 6. No rationale has been presented for why the specification fails to provide sufficient written description for strains to be used in a process for making polysaccharides selected from the group consisting of xanthan gum, alginates, gellan hum, zooglan, hyaluronic acid, and microbial cellulose (claim 5). No rationale has been presented for why the specification fails to provide sufficient written description for strains wherein the nuclease gene is integrated into a host strain selected from the group consisting of *Ralstonia eutropha, Methylobacterium organophilum, Methylobacterium extorquens, Aeromonas caviae, Azotobacter vinelandii, Alcaligenes latus, Pseudomonas*

oleovorans, Pseudomonas fluorescens, Pseudomonas putida, Pseudomonas aeruginosa, Pseudomonas acidophila, Pseudomonas resinovorans, Escherichia coli, and Klebsiella (claim 7).

The specification and examples provide clear support for the entire breadth of the claimed subject matter. Claims 1-10 meet the written description requirement.

Rejection Under 35 U.S.C. § 102

Claims 1, 2, 4, 5, 6 and 8 were rejected under 35 U.S.C. § 102(b) as anticipated by Liebl, et al., *J. Bacteriology* 174(6):1854-1861 (1992) ("Liebl"). Applicants respectfully traverse this rejection.

<u>The Legal Standard</u>

For a rejection of claims to be properly founded under 35 U.S.C. § 102, it must be established that a prior art reference discloses each and every element of the claims. *Hybritech Inc. v Monoclonal Antibodies Inc.*, 231 USPQ 81 (Fed. Cir. 1986), cert. denied, 480 US 947 (1987); *Scripps Clinic & Research Found. v Genentech Inc.*, 18 USPQ2d 1001 (Fed. Cir. 1991). The Federal Circuit held in *Scripps*, 18 USPQ2d at 1010:

> Invalidity for anticipation requires that all of the elements and limitations of the claim are found within a single prior art reference. There must be no difference between the claimed invention and the reference disclosure, as viewed by a person of ordinary skill in the field of the invention. (Emphasis added)

A reference that fails to disclose even one limitation will not be found to anticipate, even if the missing limitation could be discoverable through further experimentation. As the Federal Circuit held in *Scripps*, Id.:

> [A] finding of anticipation requires that all aspects of the claimed invention were already described in a single reference: a finding that is not supportable if it is necessary to prove facts beyond those disclosed in the reference in order to meet the claim limitations. The role of extrinsic evidence is to educate the decision-maker to what the reference meant to persons of ordinary skill in the field of the invention, not to fill in the gaps in the reference.

For a prior art reference to anticipate a claim, it must enable a person skilled in the art to make and use the invention. "A claimed invention cannot be anticipated by a prior art reference if the allegedly anticipatory disclosures cited as prior art are not enabled". *Amgen, Inc. v. Hoechst Marion Roussel, Inc.*, 314 F.3d 1313, 1354, 65 USPQ2d 1385, 1416 (Fed. Cir. 2003).

Analysis

Claims 1 and 11 were amended to further define that the bacterial strain produces a fermentation product and is genetically modified to express a heterologous nuclease gene or mutated to improve the activity of a homologous or heterologous nuclease gene, wherein the nuclease gene product is secreted into the periplasmic space or culture medium in an amount effective to degrade at least 95% of all of the nucleic acid released following lysis of the cells in less that 24 hours and reduce the viscosity of a cell lysate in a bacterial cell culture having a density of at least 50 g/l so that recovery of the product is enhanced.

Liebl teaches Staphylococcal nuclease (SNase) expression by various *C. glutamicum* strains, wherein the *C. glutamicum* transgenic strain is to be used for investigating protein export and processing. Liebl does not disclose or suggest the reduction of viscosity of a cell lysate as a direct result of secreting a nuclease into the periplasm or growth medium. Liebl does not disclose or suggest a bacterial strain for production of a fermentation product. Liebl assesses SNase expression using Oxoid plates. While the results shown in figure 6 show SNase activity, there is no teaching of an effective amount of secreted nuclease activity to reduce viscosity of a cell lysate or for production of a fermentation product. As the examples of the present application demonstrate, the **vast majority** of bacterial strains will **not** produce a nuclease in an effective amount to reduce the viscosity to facilitate product recovery, even on a laboratory scale, much less a commercial scale, although if one makes enough strains, it is possible to screen for strains that do have the desired characteristics (see Example 6, wherein cell densities of 200g/l in 20 L fed-batch cultures were used, and expressed nuclease activity was at a high enough level to adjust lysate viscosity levels that were at least comparable to those levels obtained by exogenously adding commercial BENZONASE™ to wild type cultures). Commercial scale fermentation processes require high levels of nuclease activity in order to enhance product recovery from large fed-batch cultures grown to high cell densities.

Liebl does not screen for, nor identify, any strains for production of a fermentation product that secrete nuclease in an effective amount to reduce viscosity of the cell. The assay utilized by Liebl is a qualitative, not a quantitative, assay, and would not provide the means for

one to assess the efficacy of the secreted nuclease to enhance recovery of a product from a fermenting bacterial strain.

With respect to Claims 2 and 4, there is no teaching in Liebl of a fermenting bacterial strain that is capable of growing to densities of at least 50g/L. The Examiner has failed to provide any evidence to support the notion that cell growth to at least 50g/L is an inherent characteristic of fermenting bacterial cells. There is no teaching in Leibl of an aqueous process to manufacture poly(3-hydroxyalkanoates) granule suspension. Liebl does not teach the production of any polyhydroxyalkanoate.

With respect to Claim 5, there is no teaching in Leibl of a process for making polysaccharides, and certainly not those as claimed.

With respect to claim 8, there is no teaching in Leibl to suggest an amount of nuclease that is present to degrade at least 95% of all the nucleic acid released following lysis of the cells in less than 24 hours.

The application contains examples demonstrating that one can genetically engineer known strains of bacteria with known nuclease genes to make a product as claimed. Moreover, these strains can be screened for the desired characteristics with no further information than a simple assay for nuclease activity, or the desired viscosity of the cell lysate. Example 6, demonstrates the actual isolation of products from cell lysates from an engineered, screened bacterial strain, MBX 985, and a non-engineered strain (page 16, line 19 to page 17, line 5). The engineered strain produced the same low viscosity of the cell lysate that chemical treatment was required to obtain from the non-engineered strain. Liebl does not disclose mutating and

screening; therefore the possibility that Liebl inherently discloses the claimed strains is extremely low. This is quite different from what is observed under laboratory conditions and what is disclosed by Liebl.

The Examples clearly show that a high level of nuclease expression in the constructed strains is required to generate commercial levels of product (for example polyhydroxyalkanoate), and that one must screen for these strains to identify them – although multiple strains having the desired characteristics were identified, **none** of the naturally occurring strains were sufficient.

Liebl does not show that expression decreases viscosity. Liebl does not show that a decrease in viscosity is inherent or that it is done at an appropriate level to decrease viscosity. At high volume, the cultures of Liebl wouldn't be commercially viable.

Furthermore, no product is made by Liebl. Liebl expresses nuclease only in the absence of a product such as PHA. The presence of product would certainly increase the viscosity because there is more material present. Liebl is not the same system as the present application, nor is it trying to achieve the same result. The presence of the product is what makes the present system different from Liebl. Liebl does not enable one of ordinary skill in the art to obtain a bacterial strain for production of a fermentation product by expressing a nuclease in an effective amount to reduce viscosity in order to obtain the fermentation product. The concentration of nuclease in Liebl is not disclosed. One cannot even be sure that the enzyme of Liebl is active. Nothing in Liebl allows one of ordinary skill in the art to say that the enzyme's activity will decrease the viscosity, or even that the enzyme is active in stationary phase. Therefore, claims 1, 2, 4, 5, 6 and 8 are not anticipated by Liebl.

Rejection Under 35 U.S.C. § 103

Claims 1-10 were rejected under 35 U.S.C. § 103(a) as obvious over WO 94/10289 to Greer, et al., ("Greer"), Atkinson, et al., Biochemical Engineering and Biotechnology Handbook, 2nd Edition, Stockton Press: New York, 1991 ("Atkinson") and Lee, et al., *Adv. Biochem. Eng. Biotechnol.* 52:27-58 (1995) ("Lee"), in view of Liebl or Miller, et al., *J. Bacteriology* 169(8):3508-3514 (1987) ("Miller"). Applicants respectfully traverse this rejection to the extent that it is applied to the claims as amended.

The Legal Standard

The U.S. Patent and Trademark Office has the burden under 35 U.S.C. § 103 to establish a *prima facie* case of obviousness. *In re Warner et al.*, 379 F.2d 1011, 154 U.S.P.Q. 173, 177 (C.C.P.A. 1967), *In re Fine,* 837 F.2d 1071, 1074, 5 U.S.P.Q.2d 1596, 1598-99 (Fed. Cir. 1988). To establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations. The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art and not based on applicant's disclosure. *In re Vaeck,* 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991).

The prior art must provide one of ordinary skill in the art with the motivation to make the proposed modifications needed to arrive at the claimed invention. *In re Geiger*, 815 F.2d 686, 2

U.S.P.Q.2d 1276 (Fed. Cir. 1987); *In re Lalu and Foulletier*, 747 F.2d 703, 705, 223 U.S.P.Q. 1257, 1258 (Fed. Cir. 1984). Claims for an invention are not *prima facie* obvious if the primary references do not suggest all elements of the claimed invention and the prior art does not suggest the modifications that would bring the primary references into conformity with the application claims. *In re Fritch*, 23 U.S.P.Q.2d, 1780 (Fed. Cir. 1992). *In re Laskowski*, 871 F.2d 115 (Fed. Cir. 1989). This is not possible when the claimed invention achieves more than what any or all of the prior art references allegedly suggest, expressly or by reasonable implication.

The case law has **clearly** established that the cited references **must** recite each and every element of the claims **as well as** provide to one of skill in the art the motivation to combine the cited references **and** provide one of ordinary skill in the art with a reasonable expectation of success. The references cited by the Examiner clearly do not satisfy these criteria.

Analysis

Greer

Greer describes the exogenous addition of peroxide to a cell culture. As stated in the Examples of Greer, and as stated as one of the problems addressed by the presently claimed invention, the exogenous addition of nucleases is generally known and too expensive to use for commodity fermentation products involving high cell density fermentations. Applicants are using elevated expression of nuclease instead of peroxide addition.

Liebl

Liebl describes the heterologous expression of a *Staphylococcus aureus* nuclease gene in *C. glutamicum* and the use of this transgenic system for investigating protein export in *C.*

glutamicum, as discussed above. However, there is no disclosure of possible uses for the disclosed nuclease, other than for elucidating protein export and processing mechanisms.

The Examiner has stated that "any amount excreted into the medium would result in a decrease in the viscosity of the medium and therefore an enhancement of product recovery". This is simply not true – *as demonstrated by the examples*. Compare the levels of expression of engineered strains in Table 1, all of which had been screened initially for *some* nuclease expression. Claim 1 reads an "....*effective amount* of nuclease activity to degrade nucleic acid so that *recovery of the product is enhanced*" (emphasis added). One of ordinary skill in the art of protein/product purification from protein/product producing microorganisms, will realize that an effective amount does not correlate with just "any amount of nuclease excreted into the medium", ***as demonstrated by the examples which unequivocally demonstrate the need for elevated expression***. One of skill can ascertain, without undue experimentation, what an effective amount of nuclease activity is, in view of the specification and the Examples provided therein. A level of nuclease *activity* that is "proper" can be determined by one practiced in the art of purifying product from bacterial strains, as illustrated, for example, in Table 2. Commercial scale fermentation processes require high levels of nuclease activity in order to enhance product recovery from large fed-batch cultures grown to high cell densities. Absent a teaching both to engineer then screen for very high levels of **secreted nuclease** activity, one would not obtain the claimed strain.

Miller

Miller teaches the use of a *B. subtilis* secreted nuclease for investigating "the nature of the processing of the nuclease signal peptide". Miller further characterizes the secretion of nuclease and the processing of the signal peptide from the precursor protein in *B. subtilis*. Miller speculates that the *staphylococcal* nuclease and its gene may be very useful for the development of secretion vectors for foreign proteins. There is no teaching in Miller to select for strains which secrete into the periplasm or growth medium so that recovery of a large scale product is enhanced.

Atkinson

Atkinson is a general review of biochemical and biotechnological methods and reagents.

Lee

Lee reports on production of PHAs in bacteria, and control of fermentation conditions.

The References in Combination

(a) The Prior Art Fails to Disclose or Suggest Each and Every Element of the Claims

The claims require a bacterial strain that can produce both a fermentation product and a nuclease to degrade nucleic acid in the growth medium and reduce viscosity of the cell lysate. None of the prior art discloses or suggest a genetically engineered or mutated bacterial strain that possesses these attributes. Therefore, the prior art references, individually or in combination, failed to teach or suggest each and every element of the claims as required under 35 U.S.C. § 103. *In re Fritch* 972 F.2d 1260, 23 USPQ2d 1780 (Fed. Cir. 1992); *In re Royka*, 490 F.2d 981, 180 USPQ 580 (CCPA 1974).

(b) The Prior Art Does not Provide Motivation to Combine

Contrary to the examiner's assertion, Greer, Atkinson, and Lee in view of Liebl or Miller do not provide a motivation for one of ordinary skill in the art to express a nuclease by genetic engineering to degrade nucleic acid in a growth medium for the enhanced recovery of a fermentation product. Greer states that purified preparation of nucleases are expensive. Greer lists several processes in addition to the nuclease process, e.g., heat process and precipitating by a chemical agent as unsatisfactory alternatives for reducing the viscosity of a cell lysate. Greer further discloses the peroxide process which Greer believes is better in terms of efficiency and economy.

Greer does not provide the motivation to use a genetically engineered bacteria strain because Greer states that purified preparation of nuclease is expensive. The statement that purified preparation of a nuclease is expensive **logically** leads one of ordinary skill in the art to using a process **other than** one that uses a nuclease. For example, the peroxide process disclosed in Greer is his proposed solution. To find otherwise is clearly **a typical hindsight reconstruction** that had been repeatedly discredited by the courts. *See i.e.*, *In re Dembiczak*, 175 F.3d 994, 999 (Fed. Cir. 1999); *Hodosh v. Block Drug Co., Inc.*, 786 F.2d 1136, 1143 n.5, 229 USPQ 182, 187 n.5 (Fed. Cir. 1986).

Second, even if one argued that Greer provides motivation for one of ordinary skill in the art to use a genetically engineered or mutated bacteria strain to express a nuclease, Greer certainly does not provide any motivation for one of ordinary skill in the art to genetically

engineer a bacteria strain such that the bacteria strain produces both a fermentation product <u>and</u> a nuclease for degrading nucleic acid in the growth medium for the product.

While Lee describes the various processes for the production of PHAs in bacteria, none of Atkinson, Lee, Liebl or Miller disclose or suggest using a nuclease for degradation of the nucleic acid in a cell lysate. Therefore, the prior art references, individually or in combination, do not provide one of ordinary skill in the art to genetically engineer a bacteria strain such that they express enzymes that produces a fermentation product and a nuclease that breaks down the nucleic acid in the growth medium.

Absent a suggestion to screen for very high levels of secreted nuclease, one would not be motivated to combine the references, as the applicants have done, with an expectation of success. Indeed, the prior art teaches away from the claimed invention by disclosing that one must add exogenous nuclease if one is to achieve the desired lowering of viscosity – this is the best evidence applicants can provide of why the claimed bacterial strains are not obvious: <u>those skilled in the art thought enough nuclease could not be produced by the bacteria</u>. It was only applicants that have demonstrated that one could achieve the necessary levels. The claimed bacterial strain is a secretor of an effective amount of nuclease activity. It is apparent from the Examples that the fermenting bacterial strains are for large commercial scale manufacturing of products (see Example 6, wherein cell densities of 200g/l in 20 L fed-batch cultures were used, and expressed nuclease activity was at a high enough level to adjust lysate viscosity levels that were at least comparable to those levels obtained by exogenously adding commercial BENZONASE™ to wild type cultures).

The claimed fermenting bacterial strains are selected based upon a screening process that allows one to eliminate those cells/strains that do not secrete sufficient levels of nuclease to enhance large batch product recovery. See, for example, strain MBX 985 as presented in Tables 1 and 2. Table 1 reports nuclease activity, the optical density of the culture at 600 nm, doubling time of the strain, and the percentage PHA of the bacterial cell dry weight; wherein MBX 985 retained the growing ability and PHA accumulation activities of the wild type strain (MBX 978). Based upon the PHA product accumulation and relative nuclease activity, strain MBX 985 was *selected* and grown to a cell density of 200g/L in 20 L fed-batch cultures and lysed, exhibiting the characteristics as outlined in Table 2 and described above. Nowhere does the prior art lead one skilled in the art to such a screening process.

With respect to Claims 2 and 4, there is no teaching in any of the references, singly or in combination, of a fermenting bacterial strain that is capable of growing to densities of at least 50g/L. The Examiner has failed to provide any evidence to support the notion that cell growth to at least 50g/L is an inherent characteristic of fermenting bacterial cells. There is no teaching in any of the references, singly or in combination, of an aqueous process to manufacture poly(3-hydroxyalkanoates) granule suspension. Liebl does not teach the production of any polyhydroxyalkanoate.

With respect to Claim 3, there is no teaching in any of the references, singly or in combination, of a bacterial strain which produces a polyhydroxyalkanoate to levels of at least 40% of its dry cell weight.

With respect to Claim 5, there is no teaching in any of the references, singly or in combination, of a process for making polysaccharides, and certainly not those as claimed.

With respect to claim 7, there is no teaching in any of the references, singly or in combination, of integrating a heterologous nuclease gene or a genetically modified homologous nuclease gene into a host strain selected from the group consisting of *Ralstonia eutropha, Methylobacterium organophilum, Methylobacterium extorquens, Aeromonas caviae, Azotobacter vinelandii, Alcaligenes latus, Pseudomonas oleovorans, Pseudomonas fluorescens, Pseudomonas putida, Pseudomonas aeruginosa, Pseudomonas acidophila, Pseudomonas resinovorans, Escherichia coli, and Klebsiella,* wherein the nuclease is secreted into the periplasm or growth medium resulting in an effective amount of secreted activity to degrade nucleic acid so that recovery of the product is enhanced.

With respect to claims 8-10, there is no teaching in any of the references, singly or in combination, to suggest an amount of nuclease that is present to degrade at least 95% of all the nucleic acid released following lysis of the cells in less than 24 hours; enhancing nuclease activity *via* gene expression from a modified homologous nuclease gene; or mutagenizing the bacterial strain of claim 1 in order to produce an effective amount of nuclease activity. Therefore, there is no motivation to combine the cited references.

(c) <u>The Prior Art Does not Provide a Reasonable Expectation of Success</u>

The prior art can not lead one of ordinary skill in the art to have a reasonable expectation of success. In order for one of ordinary skill in the art to successfully make or use the claimed subject matter, there at least two hurdles to overcome: (1) the co-expressing of the enzymes that

produce the product as required in the claims and the nuclease that degrades nucleic acids in the growth medium for the product, and (2) the expression of the nuclease in an effective amount for the reduction of viscosity of the growth medium. As the foregoing discussion demonstrates, the prior art references, individually or in combination, fail to teach or suggest the co-expression of the nuclease and the enzymes that produce the product listed in the claims. Furthermore, nowhere is there any teaching that would lead one to combine a heterologous gene, or regulatory sequences which enhance production of a gene, encoding a nuclease which is expressed and then secreted into either the periplasmic space or the growth medium in an amount effective to degrade nucleic acid sufficient to decrease viscosity and more economically obtain a product produced by bacterial fermentation.

The examples further demonstrate that one of ordinary skill in the art can not have a reasonable expectation of success of the claimed subject matter. *See* Examples 1-6. Indeed, just looking at the examples one realizes that most of the samples that were screened did not integrate the nuclease gene, did not secrete nuclease either into the cell culture medium or periplasmic space, and did not produce sufficient quantities of nuclease to reduce the viscosity of the cell culture medium and would therefore not have been useful.

Therefore, one of ordinary skill in the art can not have a reasonable expectation of the success of the claimed subject matter. Accordingly, claims 1-10 are not obvious over Greer, Aktinson, and Lee in view of Liebl or Miller.

Allowance of claims 1-10, and rejoinder and allowance of claims 11-23 is respectfully solicited. Claims 11-23 are related to claims 1-10 as product and process of use. Accordingly, no new search would be required should claims 1-10 be found to be allowable.

Respectfully submitted,

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